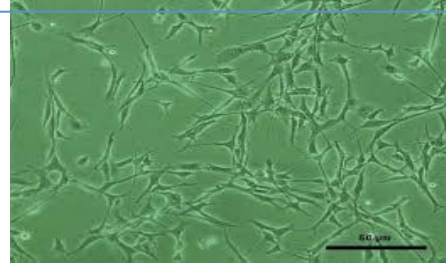

3D Human HUVECs Angiogenesis

Product Name	3D Human HUVECs Angiogenesis
Catalog	EP009
Product Format	6, 12, and 24 well
Storage	37°C



(NOTE: Other commonly used endothelial growth medium may also be used with our 3D Human HUVECs Angiogenesis, but customer need optimize conditions before using those medium.

GENERAL INFORMATION

Our 3D Human HUVECs Angiogenesis model is construct using GFP- Tagged human umbilical vein endothelial cells (HUVECs) are co-cultured with RFP- Tagged supporting cells. GFP positive capillary like tubule formation can be monitored in real time under fluorescence microscope throughout the whole process of the experiment.

Advantages:

- 1) Cells used in the 3D model are all human cells; results obtained are more relevant to human situations rather than those data from animal models, i.e. CAM et al.
- 2) The whole angiogenesis process can be monitored (from cell inoculation to the end of experiment), therefore, more crucial information can be acquired at multiple time points from a single experiment.
- 3) No need to perform post-experimental staining for endothelial markers, this is particularly important, if those markers are changed in experimental conditions involved in the studies.

The 3D Human HUVECs Angiogenesis contains all of the materials necessary to perform multiple angiogenesis assays in 6, 12, or 24 well formats. The 3D model is designed that the testing materials, i.e. compounds, conditioned media, or tissue explants, can be added into the system at any time, ranging from the onset of vasculogenesis to advanced angiogenesis. The resulting effect on tubule formation (tubular length, number of branches et al) can be monitored throughout the whole process under inverted fluorescence microscope.

Introduction:

Angiogenesis is a multistep process whereby new blood vessels develop from preexisting vasculature. Angiogenesis plays a key role in numerous physiological and pathological processes and understanding the mechanism of angiogenesis will therefore provide new approaches to the treatment of a wide range of pathologies. Angiogenesis is a complex process in which the following events are believed to play a critical role:

- Proteolytic degradation of the extracellular matrix
- Directed migration of endothelial cells
- Proliferation of endothelial cells
- Deposition of new extracellular matrix
- Formation of tubules and anastomosis of the newly formed vessels

The 3D Human HUVECs Angiogenesis Kit series products are the proprietary systems of Alphabioregen in which GFP-tagged human endothelial cells from variable vascular beds are co-cultured with RFP-tagged human supporting cells in a specially designed medium. The endothelial cells initially form small islands within the culture matrix. They subsequently begin to proliferate and then enter a migratory phase during which they move through the matrix to form threadlike tubule structures with lumens. They gradually join up (by 1 - 2 weeks) to form a network of anastomosing tubules, which closely resembles the capillary bed found in vivo.

Reagents and Materials Provided:

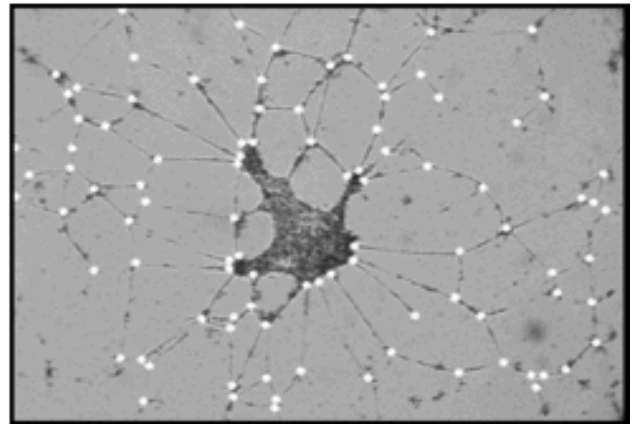
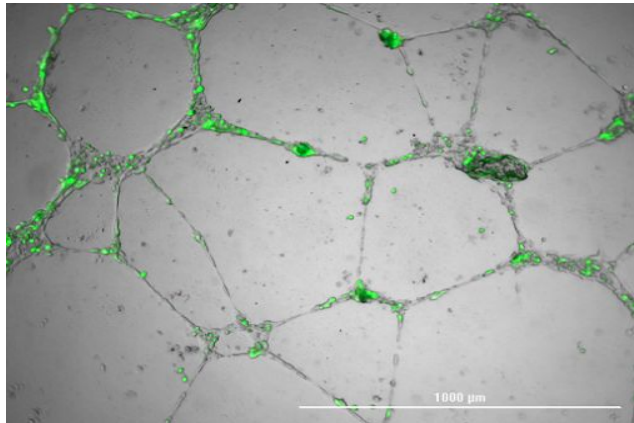
- 1 x vial of mixture of GFP-tagged HUVECs and RFP-tagged human mesenchymal supporting cells (-80°C or liquid N₂).
- 1 x 24-well AlphaBio coated plate (Room temperature) (3) 1 x 500ml of Endo-Growth Medium (4°C).

Protocol Day 1:

- Pre warm Endo-Growth Medium to 37°C in a water bath
- Accurately pipette 24ml Endo-Growth Medium into a 50ml Falcon tube
- Rapidly thaw the vial of cryopreserved cells in a 37°C water bath
- Transfer all cells gently into 24ml pre warmed Endo-growth medium
- Mix well the cells gently using a serological pipette
- Add 1.0ml of cell suspension to each well of the pre coated 24-well plate
- Make sure the cells are evenly dispersed in the wells
- Place the plate in an incubator (37°C, 5% CO₂ and humidified)

Day 2:

- Take the plate from the incubator and examine cells under inverted fluorescence microscopy (GFP positive HUVECs should sparsely and evenly distributed among RFP positive human supporting cells)
- Wash the cells one with 2 ml of PBS
- Add 2.0ml of fresh Endo-Growth medium (control) or Experimental media (Endo-Growth medium, plus pro- or anti-angiogenic reagents according to customer's needs)
- Place the plate back into the incubator. Day 4, 6, 8, 10, 12, and 14..... 13. Replace the medium every 2 days until the end of the experiments.



Recommended Coating Volume

Well	Well Area (cm ²)	Coating Volume (ml)
6	4.67	0.6
12	1.2	0.25
24	0.33	0.05